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Mating pattern and pollen dispersal in the wild olive tree (*Olea europaea* subsp. *cuspidata*)

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Abstract

In this study, the mating system, contemporary pollen flow, and landscape pollen connectivity of the wild olive tree (*Olea europaea* subsp. *cuspidata*) were analyzed in a fragmented landscape of less than 4-km diameter located in north-western Ethiopia. Four remnant populations of different sizes were investigated. Eight highly polymorphic microsatellite markers were used to genotype 534 adults and 704 embryos. We used contrasting sampling schemes and different methodological approaches to analyze the pollen flow. We observed a lower rate of inbreeding and correlated mating in the fragmented vs. the non-fragmented subpopulation. Using parentage analysis, we detected a bidirectional pollen movement across subpopulations. Pollen flow was found to be directed towards small subpopulations based on parentage and anisotropic analysis. Pollen immigration amounted to more than 50%. Although most pollination occurred within a distance of less than 200 m, longer distance pollen movements of more than 3 km were also detected. Pollen dispersal in the large and dense subpopulation was reduced, and a smaller number of effective pollen sources were detected compared to a smaller fragmented subpopulation. We obtained consistent estimates for the number of effective pollen donors (approximately 6 per mother tree) using three different methods. The average pollen dispersal distance at the landscape level amounted to 276 m while at the local level, 174 m was estimated. Bigger trees were better pollen contributors than smaller trees. We showed here for the first time that pollen dispersal in wild olive follows a leptokurtic distribution.

Keywords Contemporary pollen flow · Landscape pollen connectivity · Leptokurtic · Mating system · Microsatellites · Outcrossing

Introduction

Effective long-distance pollen movement enhances inter-population gene flow and reduces selfing or biparental in-breeding (Young et al. 1996; Wang et al. 2011; Kremer et al. 2012). Although studies in many tree species have shown that most pollination occurs within a few hundred meters, pollen

movement over more than 100-km distance has been observed (Kremer et al. 2012). Due to anthropogenic impact on the genetic structure and adaptability of forest trees, an accurate estimate of contemporary pollen flow has become very relevant (Burczyk et al. 2004). Currently, two complementary methods in terms of sampling scale and research questions can be used to infer contemporary pollen flow, i.e., indirectly using genetic structure analysis of the pollen pool or using parentage analysis (Smouse and Sork 2004; Burczyk and Koralewski 2005). How much immigrating pollen arrives in a population and how far pollen travels are major questions in gene flow studies (Burczyk et al. 2004). The spatial scale of effective pollen dispersal is a function of different biological and physical processes that affect pollen quantity, movement, and viability before and during movement (Kremer et al. 2012). The rate of pollen flow into a population of a tree species depends on the mode of pollination, isolation from the nearest conspecific tree, landscape features, the size of the studied population, and its fragmentation level (Burczyk

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et al. 2004; Smouse and Sork 2004; Sork and Smouse 2006). Numerous studies showed a high pollen immigration and fat-tailed dispersal pattern in wind-pollinated tree species (Austerlitz et al. 2004; Burczyk et al. 2004).

Long-distance pollen movement can effectively connect isolated populations. Often, natural forests exist as small remnant patches mainly due to anthropogenic influence. Therefore, besides the rate and distance of pollen movement, understanding the dynamics of pollen flow (genetic connectivity) in a landscape has become an important research question (Sork et al. 1999; Burczyk et al. 2004; Sork and Smouse 2006; Kamm et al. 2009). The success of pollen flow across the landscape depends on the size of the fragmented (sub-)populations, the distances among them, the flowering synchrony, and the wind direction (Goodell et al. 1997; Fuchs et al. 2003; Saro et al. 2014). On top of this, theoretical results indicate that intraspecific spatial aggregation also affects effective dispersal (Robledo-Arnuncio and Austerlitz 2006). Empirical data under non-fragmented and fragmented scenarios indicated the presence of equally large-scale pollen flow (White et al. 2002; Byrne et al. 2007, 2008; Hanson et al. 2008; Ismail et al. 2012). However, the effect of gene flow in connectivity studies may not be detectable due to insufficient sampling. It is very important to consider the distance between patches with meaningful pollen exchange when analyzing genetic connectivity of fragmented (sub-)populations (Anderson et al. 2010; Mona et al. 2014). Fragmentation affects a number of mating system parameters including outcrossing rate. Although a meta-analysis of mating system studies indicated the presence of high selfing and biparental inbreeding in small and fragmented populations in general (Eckert et al. 2010), there are findings that in fragmented populations outcrossing was not reduced (Young et al. 1996; White et al. 2002; Mona et al. 2014) or even enhanced (Wang et al. 2011) compared to less fragmented populations. Another mating system parameter which can be altered by population fragmentation is the degree of correlated mating events, which is the probability that sibling pairs are fully outcrossed (Ritland 2002). In a number of studies, increased correlated mating in a fragmented population was found mainly associated with a low number of reproductive trees (e.g., Lee 2000).

In this study, we analyzed *Olea europaea* subsp. *cuspidata*, which is a native tree species widely found in the dry Afromontane sub-region of the Ethiopian highlands (Bekele-Tesemma et al. 1993). It has high economic value and is very useful for rehabilitation of a fragmented landscape in this area because of long persistence of seeds and easy regeneration (Bekele 2005). The species has been exploited for at least 3000 years, mainly for farm tool preparation and cutting for fuel; large parts of these forests were cleared for agricultural land (Darbyshire et al. 2003). At present, large to small remnant populations of wild olive are only found around older monasteries and churches and at some inaccessible mountain sites; their existence is highly scattered in the open landscape (Zegeye et al. 2011). The species is poorly investigated in comparison to its Mediterranean or Saharan counterparts and possible genetic consequences of fragmentation are unknown. Therefore, the current study was carried out in a fragmented landscape of approximately 4-km circular diameter consisting of four (two big and two very small) forest fragments using highly polymorphic microsatellite markers. The objectives were to describe the mating patterns in the fragments, to estimate distance and amount of pollen immigration, and to analyze the connectivity through pollen flow. This work is the first to investigate contemporary gene flow in this subspecies. We report the dispersal kernel in a natural population of the olive tree in order to improve our understanding of how remnant forest patches are genetically interconnected and how population density affects pollen dispersal.

Materials and methods

Study species

Wild olive tree (*O. europaea* L. subsp. *cuspidata* (Wall. ex DC.)) is naturally occurring in Eastern and Southern Africa, southwestern Asia, and some parts of China (Green 2002). In Ethiopia, it is distributed from middle to higher altitudes in the dry Afromontane sub-region and reaches an average height of up to 15 m; exceptionally, it may reach up to 25 m (Bekele-Tesemma et al. 1993). The species is a diploid ($2n = 46$), wind-pollinated tree with hermaphroditic or male flowers (Besnard et al. 2008; Breton and Bervillé 2013). The flowers are whitish and the fleshy fruits are oval and of purple color (Bekele-Tesemma et al. 1993). Generally, flowers of the sub-species are morphologically similar to the cultivated forms, the proportion of staminate to hermaphroditic flowers can reach approximately 10%, and both female and male gametophytes develop synchronously (Caceres et al. 2016). Flowering time in its native range is during October and December (Cuneo and Leishman 2006). From cultivated forms and Mediterranean wild counterparts, we know that pollen production is enormous in this species. Outcrossing is further enhanced through male sterility and self-incompatibility (Besnard et al. 2000; Cuevas and Polito 2004; Breton et al. 2014). Birds are the most important seed dispersers, but also rodents, the African civet, and monkeys feed on the fruits and disperse the seeds (Cuneo and Leishman 2006; Abiyu et al. 2012, 2015).

Study area

The study was done in remnant forests located in northwestern Ethiopia (Fig. 1). This landscape is intensively used for crop cultivation and pasture land and forests in this landscape are highly fragmented. All forests are found at higher altitudes compared to the agricultural land. The study site consisted of four forests, which we call subpopulations in the following. They are located within a diameter of approximately 4 km. An intensively studied plot (ISP; 2.5 ha) was sampled in the Tara Gedam monastery forest (TG) (133 ha). This unfragmented forest is *Olea* dominated, very dense, and accompanied by other woody species (Zegeye et al. 2011). The ISP comprised approximately 100 adult *Olea* individuals per hectare.

Washaendrias (WE) (23 ha) is located around 3 km west of TG and is fragmented as only 28 *Olea* individuals per hectare were found. The remaining two subpopulations are very small *Olea*-dominated patches located less than 2 km south of TG and each is less than 4 ha in size with in total approximately 60 (Silassie, SL) and 22 (Kidanemihiret, KM) *Olea* individuals, respectively. The two bigger stands (TG and WE) are the only large populations of wild olive within 50-km distance;

however, two very small forests of not more than 50 *Olea* individuals are located around 10 km northeast of TG, but these trees were not sampled.

Sample collection, DNA extraction, and markers

A total of 534 individuals were sampled in the whole meta-population. This included 299 individuals from the exhaustively sampled ISP and 138 trees randomly sampled surrounding this plot at TG. All individuals at KM (22) and random samples from WE (48) and SL (27) were also collected. Leaf samples of these trees were taken and dried in silica gel. Each potentially mature individual sample was mapped by its GPS coordinates, and measurements of tree height and diameter at breast height (*DBH*) were recorded.

In addition, a total of 704 fruit samples were collected from 33 seed trees. Twenty of these trees were located inside of the ISP and three outside of the ISP within TG, from which we sampled 370 and 110 fruits, respectively. Ten fruit samples were collected from a tree in the farmland very closely located south of TG and eight fruits were sampled from a tree located at the mountain escarpment located east of TG. In total, 171
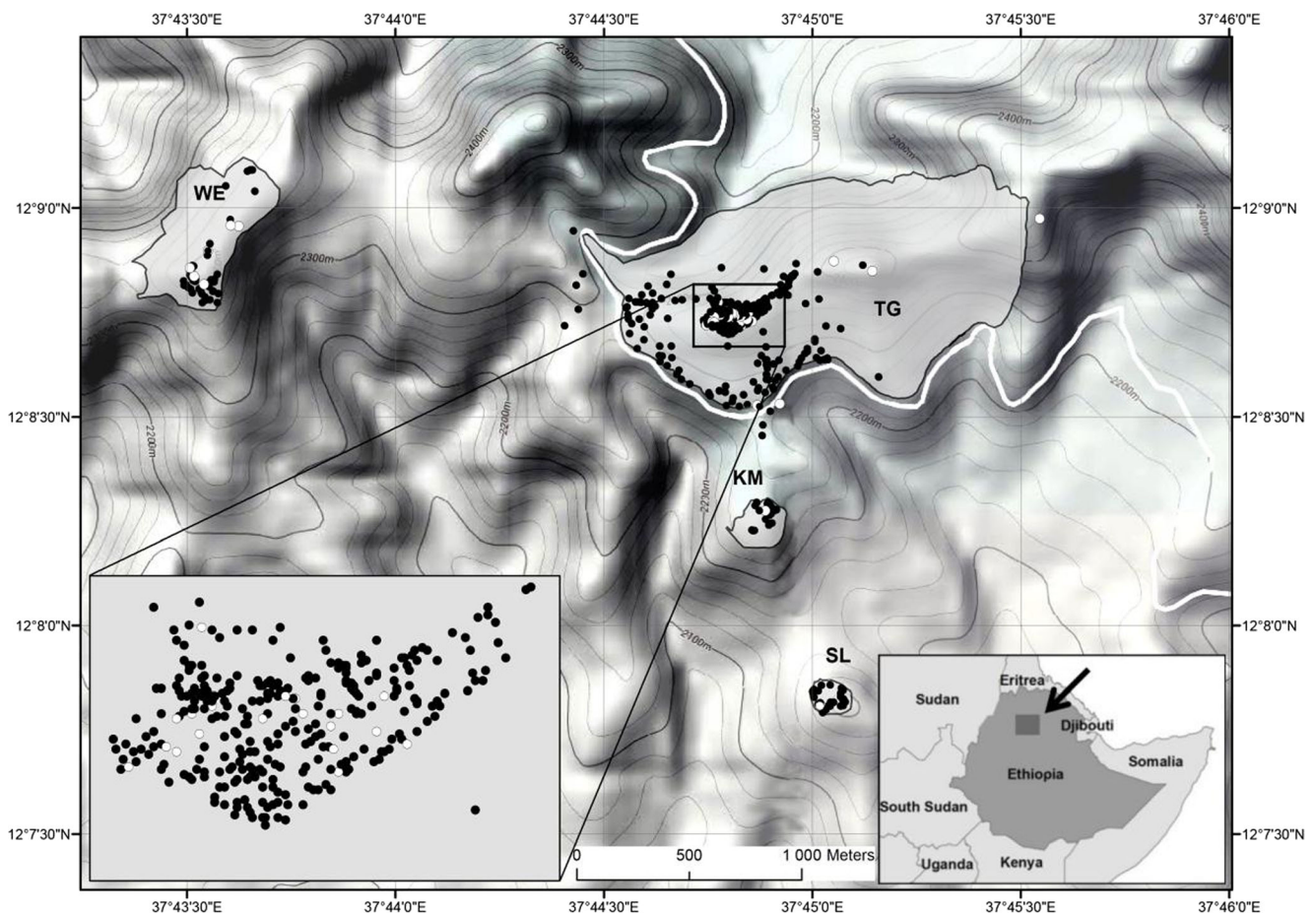
Fig. 1 Study sites [TG (including the core plot), WE, KM, SL] and distribution map of individuals sampled. Potentially flowering trees are indicated by black circles; sampled mother trees are indicated in white

fruits were sampled from six trees at WE. Nine (KM) and 24 (SL) fruit samples were collected from two trees in the remaining subpopulations (Fig. 1).

The hard seed coat (endocarp) was carefully removed from the seeds. DNA of the embryo and endosperm were extracted together by using the Qiagen DNeasy 96 Plant Kit (Qiagen, Germany). DNA from the leaf samples was extracted with the same method. Eight nuclear microsatellite markers described in Kassa et al. (2017) which are highly polymorphic with low frequency of null alleles were used for genotyping. Genotyping was outsourced to a commercial provider (ecogenics, Balgach, Switzerland) using Applied Biosystems (Foster City, CA, USA) chemistry and allele-calling tools with manual editing.

Data analysis

Genetic diversity

Genetic diversity was analyzed for adults, embryos, and sub-samples of pollen donors identified using parentage analysis using *FSTAT* v2.9.3.2 (Goudet 2001). Parameters included allelic richness (A_R) after rarefaction (Petit et al. 1998), observed (H_o) and expected heterozygosity (H_e), and inbreeding coefficient (F_{is}). For comparison, genetic diversity parameters were computed between all adults with embryos and identified pollen donors. Computation was done by using 10,000 permutations of randomized datasets. In addition, the number of private alleles in adults and embryos was computed using GenAlex v6.5 (Peakall and Smouse 2006, 2012).

Inbreeding and correlated mating

Mating system parameters were computed for the whole meta-population as well as separately for two of the subpopulations TG (unfragmented) and WE (fragmented). Computation was done based on the assumption of a mixed mating model using MLTR implementing the Expectation-Maximization (EM) algorithm (Ritland 2002). Parameters estimated were multi-locus outcrossing rate (t_m), biparental inbreeding (t_m-t_s) as the difference between the averaged single-locus estimate (t_s) and (t_m), because t_s is more biased downwards than t_m when mating between relatives occurred, and correlation of paternity (r_p). The effective number of pollen donors per mother tree (N_{ep}) was obtained as ($1/r_p$). One thousand bootstraps were computed to calculate the standard errors (SE). The latter were used to determine the probability whether t_m was significantly smaller than 1 (mean ± 1.96 [SE] < 1) and that the other parameters (t_m-t_s and r_p) were greater than 0 (mean ± 1.96 [SE] > 0). A Z-test was used to evaluate the significance of differences between the mean mating system parameter estimates of the fragmented (WE) and non-fragmented (TG) subpopulations, assuming normality for t_m,

t_m-t_s, and r_p, with normal approximation of the binomial for selfing rate (s) obtained from parentage analysis.

Paternity

Pollen donors were determined for 704 embryos considering all adult trees sampled as potential pollen sources. Paternity was assigned using the corrected likelihood equation method (Kalinowski et al. 2007) implemented in Cervus 3.0.7 (Marshall et al. 1998). Data were simulated using the reference frequency of genotypes to obtain the likelihood (LOD score) of true parents. The simulation was run by setting the number of mating events to 10,000. The proportion of candidate pollen sources sampled was assumed to be 50% of the potential pollen donors based on similar expected and observed parentage rates. The proportion of loci typed amounted to 0.981 in our dataset, and the mistyping proportion of loci was assumed to be 0.01. An inbreeding value of 7%, based on a previous analysis (Kassa et al. 2017), was used for simulation. The confidence of parentage during simulations was set at a relaxed (80%) and strict (95%) confidence interval. Pollen dispersal distances were calculated from the geographic (Euclidean) distance of the known mother to the first candidate pollen source determined. The frequency distribution of the distances between the mother trees and the respective observed pollen sources and the frequency distribution of the distances between mother trees with all potential pollen sources were compared by the Kolmogorov-Smirnov test (Sokal and Rohlf 1995). The probability of cryptic gene flow into the ISP was estimated as $1 - P_{(ex)}{}^n$, where $P_{(ex)}$ is the combined exclusion probability and n is the total number of adults sampled (Dow and Ashley 1996).

Immigration and dispersal kernel

The level of pollen flow into the ISP and parameters of the dispersal kernel were inferred using the neighborhood mating model (Burczyk et al. 2002) implemented in NM+ (Chybicki and Burczyk 2010). The approach divides parentage probabilities into three groups: (1) selfing events within the circumscribed neighborhood, (2) outcrossing events within the circumscribed neighborhood, and (3) outcrossing events with pollen sources from outside the neighborhood. The ISP was taken as neighborhood including all adults as potential pollen donors (Chybicki and Burczyk 2010). To minimize a possible sampling bias of the background allelic frequencies, these frequencies are generally better estimated from the genotypic frequencies of the embryos of the ISP as shown by Burczyk and Chybicki (2004).

Initial estimation was done by assuming random dispersal (d_p = inf) using an exponential function ($b = 1$). Subsequently, using an exponential function, the best initial values with the highest log likelihood were determined for distance d_p by

leaving it as a non-estimable parameter. With this chosen distance value, we fit the data with a simple exponential function ($b = 1$). Then, by selecting different initial values consecutively for each of the parameters (shape, kappa, and angle) and finally together, we fit the data to exponential power, Weibull, geometric, and 2Dt functions. Estimation of selection gradients for covariates was done for two growth parameters (DBH and tree height) using the standardized values under the assumption of a linear model (directional selection) using a simple exponential function.

Furthermore, KINDIST was used to estimate shape (b), scale (a), and average dispersal distance of the pollen dispersal distribution based on the expected decay with distance of correlated paternity among mother sibship pairs. To avoid inflation of the dispersal distance estimate (Burczyk and Koralewski 2005), sibs resulted from selfing of the parentage output were not included in this analysis. A threshold distance of 1750 m was set based on inspection of the correlated paternity plot to define unrelated pollen pools (Robledo-Arnuncio et al. 2007). An exponential power distribution was used to determine the dispersal function.

Pollen pool differentiation

Pollen pool differentiation (Φ_{ft}) among the pollen cloud was determined for the whole metapopulation and subpopulations TG and WE using the TWOGENER method (Smouse et al. 2001) as implemented in GenAlex v6.5 (Peakall and Smouse 2006, 2012). To compare the differentiation with an equal number of families and a comparable area size, six random families were randomly taken from the ISP. The statistical significance was tested after permutation of 999 pollen gametes among families. To reduce the effect of adult genetic structure (F_{is}) in subpopulations and the whole metapopulation, respectively, the population-specific Φ_{ft} estimate was calculated as $\Phi_{ft}/(1 + F_{is})$ according to Austerlitz and Smouse (2001), where F_{is} is the inbreeding coefficient of the adults in each subpopulation or the whole metapopulation obtained from *FSTAT* v 2.9.3.2 (Goudet 2001).

Results

Genetic diversity across generations

Estimates of genetic diversities are given in Table 1. In adults, a significantly higher number of alleles were observed than in the embryos. However, there was no significant difference in allelic richness between all adults and pollen donors identified in the parentage analysis. Adults shared 33 private alleles while for embryos only 16 alleles were found. Observed and unbiased expected heterozygosity as well as the inbreeding coefficient did

Table 1 Genetic diversity estimates across generations

	Adults	Embryos	Pollen donors	P_1	P_2
A_R	5.052	4.208	4.728	0.049	0.345
H_o	0.785	0.820	0.798	0.256	0.513
H_e	0.814	0.794	0.809	0.422	0.656
F_{is}	0.036	− 0.033	0.013	0.081	0.443

A_R, allelic richness (rarefacted for 4 individuals); H_o, observed heterozygosity; H_e, expected heterozygosity corrected for sample size; F_{is}, inbreeding coefficient; P_1 and P_2, probability value (two-sided test) after 10,000 permutations between adults and embryos, and adults and pollen donors, respectively

not differ between adults and embryos, and also not between all adults and the identified pollen donors.

Inbreeding and correlated mating

For the whole metapopulation, an outcrossing rate of 0.967 was estimated. In contradiction to our expectation, we observed a significantly smaller rate of outcrossing in the unfragmented subpopulation TG compared to WE (Table 2). Biparental inbreeding and correlated paternity were significantly higher in TG compared to WE. The effective number of pollen donors per mother tree was significantly different as shown by the correlation of paternity (Table 2).

Paternity analysis

Frequencies in each distance class of observed matings and of pairs between mother trees and their potential pollen sources are shown in Fig. 2. The combined exclusion probability for the unknown candidate parent was very high (0.999). The probability of cryptic gene flow was low 0.007. At a relaxed confidence level, paternity was assigned for 424 embryos (60%). Assignment rates were higher at TG (70%), in which sampling was more exhaustive than at WE (37%) where only few trees were sampled. A total of 214 pollen donors were identified at the landscape level with on average 6.48 pollen donors per mother tree. Out of all identified mating events, 52 (12%) resulted from self-pollination. Selfing rate was higher at TG (15%) than at WE (2%). In the ISP of all female gametes, 11% were self-fertilized, 54% were outcrossed by identified pollen donors located inside the ISP, and 35% were outcrossed by pollen donors outside the ISP.

When weighted with the number of genotypes identified, 3% of total mating events in WE were attributed to pollen donors from TG, while only 1% of the mating events in TG were attributed to pollen donors originating from WE. From the total mating events at TG, seven were attributed to pollen donors from small forest fragments

Table 2 Estimates of mating system parameters and standard errors (in brackets)

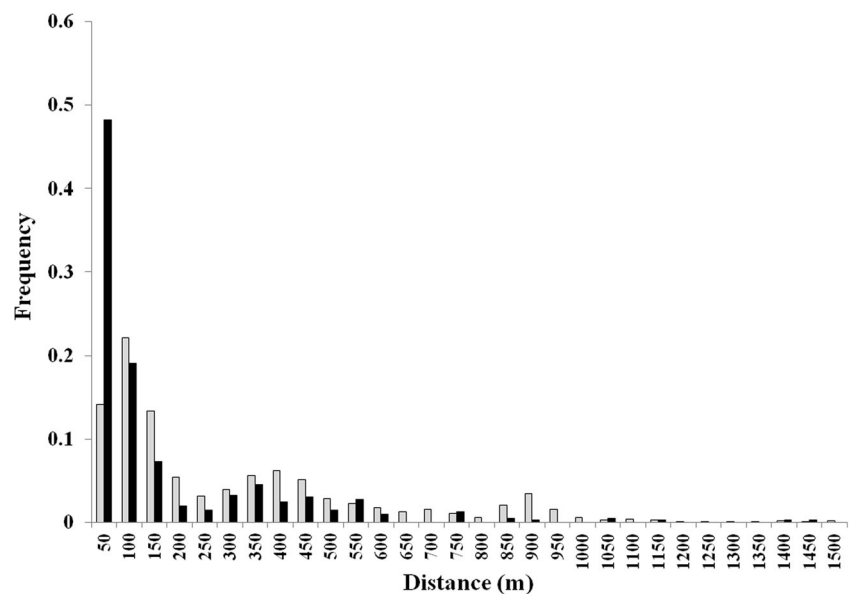| Parameter	Metapopulation	TG (dense)	WE (fragmented)	P
t_m	0.967 (0.013)*	0.949 (0.018)*	1.000 (0.000)[ns]	0.0023
$t_m\text{-}t_s$	0.170 (0.019)*	0.181 (0.027)*	0.056 (0.015)*	0.0000
$1\text{-}s$	0.877 (0.016)	0.849 (0.019)	0.984 (0.016)	0.0021
r_p	0.148 (0.029)*	0.156 (0.045)*	0.053 (0.014)*	0.0146
N_{ep}	6.76	6.41	18.87	

t_m, multi-locus outcrossing rate; $t_m\text{-}t_s$, biparental inbreeding; r_p, correlation of paternity; N_{ep}, number of effective pollen donors per mother tree estimated using MLTR; s, selfing rate estimated based on paternity analysis; P, probability of significance using Z-test between the mean estimates of the fragmented (WE) and non-fragmented (TG) subpopulations

*$p \leq 0.05$

[ns] Non-significant—$p > 0.05$, to test if $(t_m) < 1$ and $(t_m\text{-}t_s$ and $r_p) > 0$

[KM (three), SL (four)]. One mother tree in WE was pollinated by a pollen donor from SL, and one mother tree in SL was pollinated by a WE pollen source. At KM, out of nine seeds from a single mother tree, six were fertilized by pollen coming from TG. However, out of 24 seeds from a single mother tree at SL (which is relatively far > 2 km from TG), only three were fertilized by trees from TG. From all embryos of the isolated tree located south of TG, 25% were sired by pollen donors from TG, while for all embryos of the isolated tree east of TG, no pollen donors were identified.

Mating events were significantly ($P < 0.001$) distance dependent. The maximum distance of observed pollen movement was 3262 m, while on average 276 m of pollen movement was estimated and more than 75% of the pollen dispersal was observed within less than 200-m distance (Fig. 2).

Immigration and dispersal kernel

At the ISP level, we obtained high (50%) estimates of pollen immigration and 14% of self-fertilization using the neighborhood model. The outcrossing rate with mates originating from the ISP was 36%. The best fit of the data was obtained by the Weibull function (log likelihood = − 8427.53). The best fitting model suggested a fat-tailed ($b < 1$) distribution ($b = 0.7447$). The average dispersal distance was estimated to be 174 m and the direction at angle of pollen dispersal (θ_p) was 326°. The results of the other dispersal kernels are shown in Table 3. Linear selection gradient coefficients for DBH (0.77) and tree height (0.62) indicated that with increasing size, also the mating success will enhance. Average growth of the sampled trees in each remnant subpopulation can be found in Supplementary Table S1. Trees were relatively small in KM.

At the landscape level, a significant ($P < 0.001$) decline of correlated paternity of sibship pairs over distance was observed (Spearman correlation coefficients $r = − 0.181$) using KINDIST. The average pollen dispersal obtained when assuming an exponential power distribution was 417 m. The shape ($b = 0.175$) parameter estimates indicated a leptokurtic distribution of pollen dispersal.

Pollen pool differentiation

We found a moderate global differentiation among the pollen clouds of the sampled females ($\Phi_{ft} = 0.090$). The estimate was threefold higher at TG ($\Phi_{ft} = 0.0936$) than at WE ($\Phi_{ft} = 0.0335$). The estimate was translated into an effective number of pollen donors per mother tree at WE (14.9), TG (5.3), and the whole metapopulation (5.6).

Fig. 2 Frequencies in each distance class of observed matings (black bar) and between mother trees and all potential pollen donors (gray bar). Please note that the graph is truncated at 1500 m and pollen movement was observed at a distance exceeding 3000 m

Table 3 Estimates of pollen dispersal kernel parameter

Kernel family	b	a	d_p	k	θ_p	Sg dbh	sgh	Log likelihood
Weibull	0.74	145.50	174.26	− 0.59	326.2	–	–	− 8427.53
Exponential power	10.11	538.04	350.58	− 1.54	317.9	–	–	− 8498.94
Geometric	969.44	2.7×10^9	5.5×10^6	–	–	–	–	− 8551.79
2Dt	44.28	1.6×10^5	2.1×10^4	–	–	–	–	− 8551.80
Random	1 (fixed)	5.0×10^{299}	inf	–	–	–	–	− 8524.97
Exponential	1 (fixed)	2.9×10^6	5.9×10^6	–	–	0.77	0.62	− 8528.08

b, shape of dispersal kernel; a, scale of dispersal kernel; d_p, average pollen dispersal distance; k, rate parameter of directionality effect; θ_p, direction of dispersal (angle in degree from north); Sg dbh, selection gradient for DBH; sgh, selection gradient for tree height

Discussion

Compared to the findings from other studies using microsatellites in *Olea* subspecies, our estimates of genetic diversity were similar (Besnard et al. 2013) or higher (Baali-Cherif and Besnard 2005; Besnard et al. 2009; García-Verdugo et al. 2010). However, the different numbers of markers used and different populations studied as well as different sampling must be taken into account. Our relative high estimates must also be interpreted in view of historic bottlenecks caused by intensive deforestation which probably affected both SL and KM (Kassa et al. 2017). Although a loss of genetic diversity, especially measured as allelic richness, is expected in fragmented populations (Mona et al. 2014), high pollen movement such as observed in this study might have counteracted diversity loss. This was also found in other species (Saro et al. 2014).

Heterozygosity estimates were similar among generations. However, a significantly smaller number of alleles were found in embryos compared to adults, indicating that as expected not all adults contributed to the embryonic pool. Allelic richness in the subsamples of pollen donors was only slightly lower than that of all adults, which implies that the lower allelic richness in the embryos was mainly due to the analysis of only 33 maternal trees. A significant loss of allelic richness in embryos compared to the adults was also reported in Laperrine's olive (Besnard et al. 2009). Our observation of an excess of heterozygotes in the embryos ($F_{is} = − 0.036$) in comparison to the adults ($F_{is} = 0.033$) is only tentative as the difference between these cohorts was not significant ($P < 0.08$). Nevertheless, the latter finding is probably the result of predominant outbreeding due to random mating from unrelated pollen sources and relatively isolated, distant maternal trees.

Breton et al. (2014) showed that a self-incompatibility system exists in olive cultivars, and the authors suggested a sporophytic incompatibility system with several differently dominant S-alleles which prevent selfing and mating among related individuals. Moreover, effective pollen dispersal in *Olea* by wind may have further enhanced the outcrossing rate (Pinillos and Cuevas 2009). However, in rare cases, selfing occurs in olive cultivars (Mookerjee et al. 2005). Although this information cannot be directly transferred to *O. europaea* subsp. *cuspidata* and bottlenecks may have erased some of the S-alleles, a high outcrossing rate was to be expected. The outcrossing rate obtained with MLTR averaged over the whole metapopulation was high (0.967), and we obtained a moderate level of biparental inbreeding (0.170). Using individual parentages, we estimated a selfing rate of 12% for the whole metapopulation. When using the neighborhood model, the selfing rate was estimated as 14%. We argue that the latter estimates are of high biological relevance, as both the mother trees and their effective pollen sources were genotyped. In addition, the MLTR procedure does not consider the presence of mismatching loci between the mother and seed genotypes, and under this condition, it categorizes the selfed individuals into biparental inbreeding.

In contrast to the expectation (high level of selfing in small and fragmented populations; Eckert et al. 2010), outcrossing was significantly higher in the fragmented subpopulation WE in comparison to the larger and dense subpopulation TG. In most previously analyzed small fragmented populations, low outcrossing was due to a small number of effective pollen donors (Murawski and Hamrick 1991; Lee 2000). However, a higher effective number of pollen donors per mother tree (lower correlated paternity) were detected in the fragmented WE, indicating no limitation on mate availability. Higher pollen flow into the fragmented subpopulation could be a reason for the enhanced outcrossing rate at WE. The effect of canopy cover at TG could be another reason for the contrasting result of the outcrossing rate as a closed canopy cover can prevent effective pollen movement (Millerón et al. 2012).

The estimated effective number of pollen sources per mother tree in the metapopulation amounted in all three different methodical approaches to very similar estimates: 6.76 [MLTR], 6.48 [parentage analysis], and 5.56 [TWOGENER]. These estimates are within the lower range of wind-pollinated tree species (Smouse and Sork 2004). The estimate was unexpectedly high at WE compared to TG. A higher number of flowering trees at WE can be assumed as 56% of the sampled trees were involved in pollination, while at TG, this proportion was only

39%. Spatially restricted pollen dispersal at TG as indicated by high pollen pool heterogeneity might have contributed to the differences between the two subpopulations. In addition, variation in flowering synchrony and in male fertility between the subpopulations might have contributed to our observation (Dyer and Sork 2001; Robledo-Arnuncio et al. 2004; Bittencourt and Sebbenn 2007).

Pollen immigration into a studied area depends on several factors such as dispersal mechanism, phenology, size, and the level of isolation (Burczyk et al. 2004). Generally, pollen flow is very high when the studied trees are part of a continuous population. We investigated a circumscribed neighborhood of 2.53 ha in the center of a continuous olive forest. When applying the neighborhood model, we obtained a 50% immigration rate into this neighborhood; however, when the paternity was analyzed, a much lower rate (35%) was obtained, which was not affected by cryptic gene flow (0.7%). The results from the former may reflect reality better because parentage analysis was based on a relaxed paternity assignment which could overestimate the rate of non-immigrants, while this is taken into account in the probability models. A comparable result was obtained in *Oenocarpus bataua* where a 28% higher immigration rate was estimated using the neighborhood method compared to the parentage analysis (Ottewell et al. 2012).

Mating was non-random. Pollen movement was frequent within a small radius (< 200 m), which is in agreement with results observed in other wind-pollinated tree species (Robledo-Arnuncio et al. 2004; Bittencourt and Sebbenn 2007, 2008; Pluess et al. 2009). A pollen dusting experiment in the Mediterranean olive indicated that higher concentrations of pollen can be found within a short radius from the emitting source. However, Pinillos and Cuevas (2009) found out that pollen grains can cross more than 10 km. In our experiment, we detected pollen movement up to 3.26 km (the max. distance of all sampled trees was 3.46 km). This observation is similar to that of Besnard et al. (2009) who—also employing a paternity analysis—detected an effective pollen movement of approximately 2 km in a highly fragmented Laperrine's olive population.

The occurrence of bidirectional long-distance pollen movement connected all parts of the metapopulation, implying that pollen movement can counteract a loss of genetic diversity in isolated forest stands. Similar findings were also reported in other studies at a fragmented landscape level (White et al. 2002; Wang et al. 2011; Mona et al. 2014). A high pollen movement from a dense and large to a fragmented and small subpopulation was expected based on previous findings (White et al. 2002; Bacles and Ennos 2008). Indeed, also our results support this general picture; however, the number of effective pollen donors (pollen limitation) detected in each subpopulation does not explain our results. The predominant wind direction in northwestern Ethiopia is from north to south (see Annual Climate Bulletin under http://www.ethiomet.gov.

et), which does not support our finding of an anisotropic pollen movement. However, local wind directions might still affect the predominant gene flow from TG to WE. We suggest that major factors affecting anisotropy could be a contrasting tree density and/or canopy cover in each subpopulation. For instance, Dyer and Sork (2001) reported a significant negative correlation of the pollen pool diversity with tree density. Our estimate of more than a threefold inflated genetic differentiation of the pollen pool at TG compared to WE, even after accounting for the size of the population, adult genetic structure, and number of mother trees, supports our suggestion. Pollen pool differentiation among all subpopulations ($\Phi_{ft} = 0.090$) was very similar as in other wind-pollinated tree species (Dyer and Sork 2001; Bittencourt and Sebbenn 2008). Nevertheless, the lack of data on local wind direction and our non-exhaustive sampling at WE prevented a detailed comparison of pollen immigration under the two fragmentation scenarios.

Pollen dispersal was modeled at local and landscape levels using two methods (NM+ and KINDIST) which are designed for two different sampling schemes (Smouse and Sork 2004). Both scales resulted in a fat-tailed ($b < 1$) dispersal, which indicates a higher probability of long dispersal events in comparison to a simple exponential function. Similarly, a number of studies on tree species with different pollination behaviors and densities indicated that pollen dispersal follows from a slight to a strong fat-tailed dispersal curve (Austerlitz et al. 2004; Oddou-Muratorio et al. 2005; Robledo-Arnuncio and Gil 2005; Wang et al. 2010; Ramos et al. 2016). The dispersal kernel was more strongly fat tailed at the landscape than at the local level, although using two different approaches with different functions. This is consistent with the expectation from two contrasting sampling scales and our observation of effective pollination across the fragments using individual parentage. Slower decay of long-distance pollen dispersal in the landscape will maintain genetic connectivity of fragmented subpopulations. Only two parametric functions (Weibull and exponential power) were effective in modeling of the dispersal and have resulted in plausible estimates of the dispersal distance. These two functions are also mentioned as powerful to model dispersals with a high probability of long-distance events (Austerlitz et al. 2004).

At the landscape level, the average pollination distance using parentage analysis (276 m) was lower than that using KINDIST (417 m). The KINDIST method is sensitive to the presence of selfing and unknown pollen sources in the dataset; therefore, we removed all data resulting from selfing. However, the inflation due to unknown pollen sources remains unclear. Therefore, we consider the parentage estimate to be more reasonable. At the local level, the estimated average pollen dispersal distance (174 m) modeled with NM+ was lower than that at the landscape level. This is to be expected as modeling was based on samples taken only from the ISP as a

small neighborhood area with a high rate of immigration. Moreover, our findings were according to the restricted pollen movement observed in the ISP. An indirect estimate of the gene dispersal (220 m) obtained from spatial genetic structure analysis using exclusively adult trees at the landscape level (Kassa et al. 2017) was lower than contemporal estimates at the same spatial scale using parentage analysis or KINDIST. We presume that the seed dispersal in *Olea europaea* subsp. *cuspidata* is more limited than pollen dispersal. Hence, the estimate of the historic gene flow underestimates the pollen flow and differences between historic and contemporary pollen movement are not so pronounced. Model predictions on the reproductive success using individual DBH and height as linear covariates indicated that bigger trees were more successful pollinators. This can be explained by their higher pollen production, higher point of pollen emission, and/or a shifted sexual function. Similarly, other studies found a positive association between size of tree and mating success (Oddou-Muratorio et al. 2005; Saro et al. 2014).

In conclusion, we observed long-distance pollen dispersal and high outcrossing in this subspecies. This is in accordance with previous results when studying historic gene flow (Kassa et al. 2017) which could effectively maintain genetic diversity in (sub-)populations in a fragmented landscape. Even small church forests distributed throughout middle and high elevations in Ethiopia very likely contribute to the maintenance of a diverse gene pool in *Olea*. The genetic system of this tree species is obviously adapted to fragmentation and occurrence at low density.

Data archiving statement   All data will be deposited in DRYAD upon acceptance for publication.

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